

Appl. No. 09/910,432
Amndt. dated July 21, 2003
Reply to Notice to File Corrected Application Papers of May 22, 2003

PATENT

Amendments to the Specification begin on page 3 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 7 of this paper.

Remarks begin on page 14 of this paper.

Amendments to the Specification:

Please replace the paragraph beginning at page 5, line 3, with the following:

--Figure 1 provides a schematic representation the components used in the invention (G3R7 = SEQ ID NO:1).--

Please replace the paragraph beginning at page 5, line 16, with the following:

--The present invention provides a component-based system for selective, persistent, delivery of imaging agents, genes or other therapeutic agents. Individual features for the compositions can be selected by designating desired components in bedside formulations. Additionally, imaging and specific targeting moieties are provided on separate negatively charged backbones which will form a non-covalent ionic association with a positive backbone. By placing these components on a negatively charged backbone, the invention obviates the need for attaching components in precise locations on a positive backbone as employed in other strategies (increasing complexity and expense and decreasing efficiency to a level that no successful combination has yet been reported due to steric limitations). Further understanding of the invention is provided with reference to Figure 1. In this figure, the components are shown as (1) a solid backbone having attached positively charged groups (also referred to as efficiency groups shown as darkened circles attached to a darkened bar), for example (Gly)_{n1}-(Arg)_{n2} (SEQ ID NOS:2-7) (wherein the subscript n1 is an integer of from 3 to about 5, and the subscript n2 is an odd integer of from about 7 to about 17) or TAT domains; (2) a short negatively charged backbone having attached imaging moieties (open triangles attached to a light bar); (3) a short negatively charged backbone having attached targeting agents and/or therapeutic agents (open circles attached to a light bar); (4) an oligonucleotide, RNA, DNA or cDNA (light cross hatched bar); and (5) DNA encoding persistence factors (dark cross hatched bar). Figure 2 illustrates various examples of multicomponent compositions wherein the groups are depicted as set out in

Figure 1. For example, in Figure 2, a first multi-component composition is illustrated in which a positively charged backbone has associated an imaging component, a targeting component, an oligonucleotide and a persistence factor. A second multi-component composition is illustrated which is designed for diagnostic/prognostic imaging. In this composition the positively charged backbone is complexed with both imaging components and targeting components. Finally, a third multi-component system is illustrated which is useful for gene delivery. In this system, an association complex is formed between a positively charged backbone, a targeting component, a gene of interest and DNA encoding a persistence factor. The present invention, described more fully below, provides a number of additional compositions useful in therapeutic and diagnostic programs.--

Please replace the paragraph beginning at page 9, line 5, with the following:

--In a particularly preferred embodiment, the positively charged backbone is a polypeptide having branching groups (also referred to as efficiency groups) comprising -(gly)_{n1}-(arg)_{n2} (SEQ ID NOS:8-18), HIV-TAT or fragments thereof, in which the subscript n1 is an integer of from 0 to 20, more preferably 0 to 8, still more preferably 2 to 5, and the subscript n2 is an odd integer of from about 5 to about 25, more preferably about 7 to about 17, most preferably about 7 to about 13. Still further preferred are those embodiments in which the HIV-TAT fragment has the formula (gly)_p-RGRDDRRQRRR-(gly)_q (SEQ ID NO:19) or (gly)_p-YGRKKRRQRRR-(gly)_q (SEQ ID NO:20) wherein the subscripts p and q are each independently an integer of from 0 to 20 and the fragment is attached to the backbone via either the C-terminus or the N-terminus of the fragment. Preferred HIV-TAT fragments are those in which the subscripts p and q are each independently integers of from 0 to 8, more preferably 2 to 5.--

Please replace the paragraph beginning at page 9, line 17, with the following:

--In another particularly preferred embodiment, the backbone portion is a polylysine and positively charged branching groups are attached to the lysine sidechain amino groups. The polylysine used in this particularly preferred embodiment can be any of the commercially available (Sigma Chemical Company, St. Louis, Missouri, USA) polylysines such as, for example, polylysine having MW > 70,000, polylysine having MW of 70,000 to 150,000, polylysine having MW 150,000 to 300,000 and polylysine having MW > 300,000. The appropriate selection of a polylysine will depend on the remaining components of the composition and will be sufficient to provide an overall net positive charge to the composition and provide a length that is preferably from one to four times the combined length of the negatively charged components. Preferred positively charged branching groups or efficiency groups include, for example, -gly-gly-gly-arg-arg-arg-arg-arg-arg (-Gly₃Arg₇) (SEQ ID NO:1) or HIV-TAT.--

Please replace the paragraph beginning at page 22, line 8, with the following:

--The following components are prepared:

1. a positively charged backbone composed of polylysine with Gly₃Arg₇ (SEQ ID NO:1) linked via the side chain amino terminus of Lys to the carboxy terminus of Gly₃Arg₇ (SEQ ID NO:1) at a degree of saturation of 20%. A solution is prepared of the backbone moiety at a concentration of 1.5 mg/mL in phosphate buffered saline (PBS).
2. cDNA expressing blue fluorescent protein under the control of a cytomegalovirus (CMV) promoter is prepared and used at a 0.5 mg/mL concentration in PBS.
3. a dextran- DOTA- gadolinium complex (see, Casali, et al., *Acad. Radiol.* 5:S214-S218 (1998)) is used at a 1:2 dilution in PBS.--

Please replace the paragraph beginning at page 23, line 6, with the following:

--The following components are prepared:

1. a positively charged backbone composed of polylysine with Gly₃Arg₇ (SEQ ID NO:1) linked via the side chain amino terminus of Lys to the carboxy terminus of Gly₃Arg₇ (SEQ ID NO:1) at a degree of saturation of 20%. A solution is prepared of the backbone moiety at a concentration of 1.5 mg/mL in phosphate buffered saline (PBS).
2. cDNA expressing herpes simplex virus thymidine kinase gene under the control of a cytomegalovirus (CMV) promoter is used at a 0.5 mg/mL concentration in PBS.
3. dextran- DOTA- gadolinium complex is used at a 1:2 dilution in PBS.
4. Conjugate Fab fragment specific for desired tumor antigen at a 5% saturation rate to dextran of size range and concentration in PBS selected to afford 1:2 negative charge ratio relative to component "2" above.--

Please replace the paragraph beginning at page 24, line 9, with the following:

--In this example a 6-well plate was used to evaluate one iteration of the component-based strategy. The positively charged backbone was assembled by conjugating -Gly₃Arg₇ (SEQ ID NO:1) to polylysine 150,000 via the carboxyl of the terminal glycine to the free amine of the lysine sidechain at a degree of saturation of 18% (i.e., 18 out of each 100 lysine residues is conjugated to a -Gly₃Arg₇ (SEQ ID NO:1)). The resultant backbone was designated NUNU-01.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 9, at the end of the application.